Effect of Endocervical Specimen Adequacy for Detection of

*Chlamydia trachomatis* Using the APTIMA COMBO 2® Assay


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ABSTRACT

Six-hundred and one endocervical specimens were analyzed for *Chlamydia trachomatis* using APTIMA Combo 2 and evaluated for columnar epithelial cell adequacy by direct fluorescent-antibody staining. With 5.5% positive adequate and 7.8% positive inadequate specimens (P=0.27), the study suggested no difference in positivity due to specimen adequacy using this amplified technology.
*Chlamydia trachomatis* (CT) is the most common bacterial sexually transmitted disease in the United States (7). In women, symptoms of CT infection are usually mild or absent (15). The Centers for Disease Control and Prevention (CDC) has recommended widespread screening among women in an effort to control this epidemic (3). Many improvements in test technologies have evolved, including nucleic acid amplification tests (NAATs) (2,5,6,8,14,16). However, since chlamydiae are intracellular organisms which infect columnar epithelial cells, inadequate specimens with little or no columnar cells have substantially impacted the sensitivity of testing for *C. trachomatis* antigens (9,11). The value of increased sensitivity when using amplified testing to overcome inadequate specimens has not been confirmed in other studies with older NAATs (1,9-13,18). As a result, CDC has recommended monitoring of columnar cell content in endocervical specimens to assess specimen quality (4).

This study assessed whether specimen adequacy, based on the traditional presence or absence of columnar epithelial cells, significantly affected the detection of CT by a more recent NAAT, the APTIMA Combo 2 assay (Gen-Probe, Incorporated, San Diego, CA). We believed the increased sensitivity of this second generation amplification test, APTIMA Combo 2, could potentially overcome the necessity to measure cellular adequacy.

Six-hundred and one specimens were collected from women at 2 family planning clinics, 2 STD clinics, and a community health center, (Sites A, B, and C). Asymptomatic and symptomatic females who presented at the centers were eligible based on age criteria, 14-25 years (17).
Following removal of exocervical mucus, two endocervical swabs were rotated simultaneously during collection. One swab was placed in an APTIMA swab transport tube (Gen-Probe, Incorporated, San Diego, CA). The second swab was used to prepare a Microtrak slide, for determining cellular adequacy (Trinity Biotech, Jamestown, NY). Endocervical swab specimens were tested at the Wyoming Public Health Laboratory (WPHL) by APTIMA Combo 2 assay, (Combo 2), the NAAT currently used by WPHL. Slides were identity unlinked and forwarded to Johns Hopkins University, Baltimore, MD, for evaluation by direct fluorescent-antibody (DFA) staining.

Combo 2 was performed on 601 endocervical swab specimens according to assay instructions. Specimens testing positive initially, were retested a second time. Additionally, since the WPHL was participating in a separate, but concurrent regional CT study, aliquots of positive specimens were sent for testing with the “standalone” APTIMA CT assay (Gen-Probe, Incorporated, San Diego, CA), which uses an alternative and different gene target.

Slides were stained with a fluorescein-conjugated monoclonal antibody (Kallestad, Chaska, MN) and read by epifluorescence microscopy by medical technologists proficient in DFA microscopy. All 601 slides were assessed for the presence of chlamydiae elementary bodies, columnar epithelial cells, and erythrocytes. According to criteria set forth by Kellogg et al., a specimen was considered to be adequate on a cellular-component basis if it contained any columnar epithelial cells, with or without other cells (9-11).

Based on slide evaluation, 422 (70%) were graded as adequate and 179 (30%) as inadequate. A total of 37 (6%) specimens were positive by Combo 2. Of 422 adequate
specimens, 23 (5.5%) were CT positive by Combo 2. Of 179 inadequate specimens, 14 (7.8%) were CT positive by Combo 2 (Table 1). No significant difference in positivity rate between adequate and inadequate specimens was found (P=0.27). Only 7 of 601 (1.2%) were DFA slide positive, demonstrating typical fluorescent elementary bodies. These 7 were included in the 37 Combo 2 positive results. There were no discordant specimens in which Combo 2 was negative and DFA was positive. All 37 (100%) Combo 2 positives repeated positive. Thirty-four aliquots of the Combo 2 positive specimens were available and sent for testing with the “standalone” APTIMA CT assay. All 34 aliquots confirmed as positive for CT by the APTIMA CT assay. Remnant sample was unavailable for 3 of the 37 Combo 2 positive specimens.

Specimen adequacy by clinic site averaged 70%. When examining positivity rate of adequate vs inadequate groups within individual sites, numbers in each category were too small for statistical analyses. Site C routinely uses a large proctoscopic type swab for a more thorough removal of exocervical mucus, exposing more of the cellular components. There was no increase in specimen adequacy resulting from this practice.

The accepted definition of adequacy has previously been based upon columnar epithelial cell presence (1,9,10,18). However, presence of erythrocytes (RBCs) may indicate cervical friability, a common finding in CT infection (9,18). Given this scenario, the study looked at the additional presence of RBCs in adequate and inadequate groups. There was no significant change in positivity within groups containing RBCs or those without RBCs (P=0.28, P=0.48).

The use of two swabs rotated simultaneously during collection eliminated any bias resulting from one swab depositing the entire sample on the first testing platform vs the
second, i.e. NAAT vs DFA slide. Additionally, this collection method resulted in a total sample positivity of 6% (37/601), which is similar to the general 6% prevalence rate in the current population.

CDC has suggested supplemental testing after an initial positive NAAT screening in certain cases and in populations with a low prevalence of infection (4). One such approach suggested by CDC involves repeating the original test on the original specimen. In this study, all 37 positive Combo 2 specimens were repeat positive using the original test and original specimen, thus “confirming” positive results by repeat testing. Another CDC option suggested additional testing of the original specimen with a different NAAT or one that uses an alternative target or format. Of the 34 Combo 2 samples with sufficient quantity for allocation and testing with the “standalone” APTIMA CT assay, all 34 (100%) verified as positive with this alternative target method. Verification of adequate and inadequate positive specimens using both approaches further supported the finding of this study, suggesting specimen adequacy confirmation may not be necessary, when using the APTIMA Combo 2 assay, even in this low prevalence population.

Our results differed from earlier studies with amplification tests, PCR (10,18) and LCR (12), which concluded cellular adequacy was a determining factor when testing for the presence of \emph{C. trachomatis}. We were able to detect CT even when the specimen was graded as inadequate, based on the same measurement of cellular adequacy.

A potential limitation of the finding of no statistical difference in positivity between specimens graded adequate vs. inadequate could be due to lack of enough statistical power due to the small sample size. However, we feel our results point to the general
conclusion that measurement of cellular adequacy may not be required when detecting *C. trachomatis* with this NAAT assay.

In conclusion, the study suggested that cellular adequacy of endocervical specimens, when testing for *Chlamydia trachomatis* using the APTIMA Combo 2 assay, did not appear to influence positivity of the results. Regardless, submission of the best possible cervical specimen should always be a priority for clinicians who obtain endocervical samples for the diagnosis of CT infection.
We thank the study participants, the site providers who collaborated in the collection process, and the Montana Public Health Laboratory for the additional testing on positive aliquots as part of the concurrent Region VIII study. Also, thank you to Yu-Hsiang Hsieh, Johns Hopkins University, for his statistical analyses. Financial support was provided by Gen-Probe, Incorporated.
REFERENCES


American Society of Clinical Pathologists. Chicago, IL.


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### TABLE 1. Chlamydia Positivity in Adequate versus Inadequate Specimens (n = 601)

<table>
<thead>
<tr>
<th>Adequacy</th>
<th>Specimens tested no. (%)</th>
<th>APTIMA Combo 2 positive</th>
<th>APTIMA Combo 2 negative</th>
<th>Positivity rate(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate</td>
<td>422 (70%)</td>
<td>23</td>
<td>399</td>
<td>5.5%</td>
</tr>
<tr>
<td>Inadequate</td>
<td>179 (30%)</td>
<td>14</td>
<td>165</td>
<td>7.8%</td>
</tr>
<tr>
<td>Total</td>
<td>601</td>
<td>37(^a)</td>
<td>564</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

\(^a\) Aliquots from 34 Combo 2 positive specimens were available for confirmatory testing.

All 34 confirmed positive.